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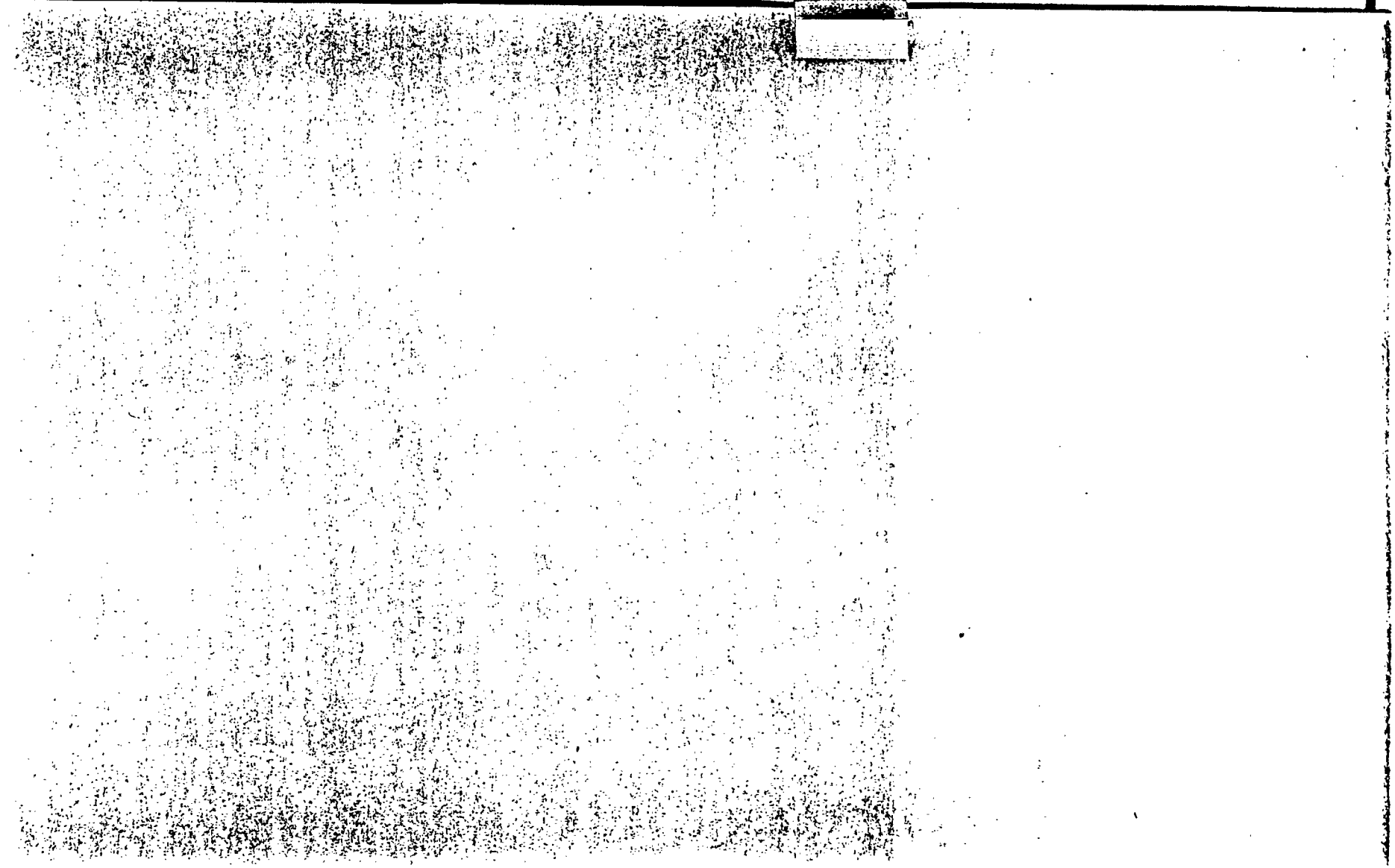
PROJECT 3



PROJECT 3

P. HAMOSH M.D.

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8585

Application for Research Grant
(Use extra pages as needed)

Date: 6/30/75

1. Principal Investigator (give title and degrees):
Jean Wrathal, Ph.D. Assistant Professor of Anatomy

2. Institution & address:
Georgetown University
37th & O Sts., N.W.
Washington, D.C. 20007

3. Department(s) where research will be done or collaboration provided:
Anatomy and Physiology

4. Short title of study:
SMOKING AND LUNG DEVELOPMENT: A PROGRAM PROJECT.
PROJECT #3, SMOKING AND THE CELLULAR AND EMBRYOLOGICAL ASPECT OF LUNG DEVELOPMENT

5. Proposed starting date: 1/1/76

6. Estimated time to complete: 5 years

7. Brief description of specific research aims:

SMOKING AND CELLULAR AND EMBRYOLOGICAL ASPECTS OF LUNG DEVELOPMENT.

In this project, the effect of smoking and other environmental factors on the growth and development of isolated lung cells will be studied. The embryological approach is two fold: 1) to produce by manipulation of the embryo and the neonate aberrant patterns of airway development and then study the effect of smoke on the adult; and the reverse 2) to study the effect of perinatal smoke exposure on devlopment of lung structure (morphometric analysis).

PROJECT 3

SMOKING AND THE CELLULAR, AND EMBROYOLOGICAL ASPECTS OF LUNG DEVELOPMENT

General Background:

In project 3 we propose a broad exploration of possible effects of cigarette smoke on: 1) different cell types in the lung; 2) lungs with different morphometric characteristics and 3) on lungs at different stages of development.

Pulmonary emphysema is characterized by destruction of alveolar walls consisting of epithelial, endothelial and interstitial cells. An increased incidence of emphysema has been found in cigarette smokers (1). Yet at present there is no information on the direct effect (if any) of cigarette smoke on the individual cell types comprising the alveolar wall. One of these, the alveolar type II epithelial cell (granular pneumocyte) appears to be intimately involved with the synthesis of surfactant (2,3,4), the substance that prevents collapse of the lung at low air volumes. Evidence suggests that type II cells also give rise to the type I epithelial lining cells (5,6). A second cell type, the alveolar interstitial cells, are the source of collagen and elastic fibers (7) that contribute to the overall elastic properties of the lung. In addition, the interstitial cells play a major role in post-natal alveolar development (8,9,10). Besides mediating transport to and from the blood, the pulmonary endothelial cells actively metabolize a number of vasoactive substances (11) that affect the organism as a whole. Lipoprotein lipase, the enzyme required for processing of plasma cylomicrons is present at or near the plasma membrane of endothelial cells (12) including those of the lung (13). Alteration inthe activity of this enzyme during development and as a result of exposure to cigarette smoke will be studied in Projects 2 and 3 of this program project.

It is now possible to isolate relatively pure populations of Type II cells (14) and the feasibility of studying these cells grown in culture has recently been demonstrated (15). Several groups have established cultures of functional active endothelial cells (e.g., 16,17,18). Although cultures of pulmonary endothelial cells have not yet been established, the existence of specific "markers" such as the enzyme that converts angiotensin I to angiotensin II (19) would allow identification of pulmonary endothelial cell in culture. Fibroblast cultures that synthesize and secrete procollagen are now routinely established from, for example, human skin biopsies (20) and the in vitro synthesis of collagen by minces of lung tissue has been studied (21).

Therefore, we propose to examine the cellular basis of altered lung function associated with cigarette smoke and other environmental agents, utilizing isolated, functional alveolar cells maintained in cell culture.

Epidemiologic studies indicate that environmental and genetic factors influence the genesis of chronic obstructive lung disease. For example, there is an additive effect of smoking and exposure to "traffic pollution" in the development of disease (22). A clearcut example of the influence of genetic factors is the congenital deficiency in alpha-antitrypsin which results in a predisposition to emphysema that is exacerbated by cigarette smoking (23). The results of twin studies (24) imply that other more subtle, genetic factors may also have an appreciable effect. In the Report of the Surgeon General (25), the question is posed, "whether lesser degrees of genetically identifiable susceptibility interact with cigarette smoking to account for a significant proportion of the problem."

Experimental genetics in humans is not feasible and no animal models have yet been developed to investigate possible genetic factors in the genesis of chronic obstructive lung disease. However, the existence of many inbred (isogenic) strains of the mouse in which structural and/or functional variants of the lung may be found makes such an approach feasible. Morphometric variants are of prime interest, as shown by the work of Lynne Reid's group (26) which correlates the number of bronchial divisions with the development of early emphysema. Indeed, the hypothesis is posed in the general introduction to this program project that "certain patterns of lung geometry (may) predispose to accelerated pathogenesis of obstructive lung disease." To test this hypothesis we propose to seek congenital variants of lung geometry (number of different bronchial divisions) in different mouse strains and/or experimentally induce such variants, and study the effects of cigarette smoke on these mice.

Environmental influences during embryonic development, or during the extensive (27) post-natal maturation of the lung may, by altering the adult lung, also alter the susceptibility of this organ to environmental "insult" in later life. One such influence could be maternal smoking during pregnancy and/or post-natal maturation. Smoking has been implicated as a cause of increased infant mortality and morbidity (28) but little information is available on the effect of cigarette smoke on the developing lung. Therefore, we also propose to study the effects of cigarette smoke and other environmental factors on the morphological and morphometric development of the mouse lung.

Principal Aims:

1. To study the effects of cigarette smoke on the growth and specific functions of isolated cell types from the alveolar wall grown in culture.

2. To determine whether mice with congenital or experimentally induced variations in lung morphometry respond differently to chronic inhalation of cigarette smoke.

3. To determine whether cigarette smoke influences pre or post-natal lung development and thus leads to altered lung properties.

General Experimental Design:

1. The animal model will be the laboratory mouse. The BALB/C strain will be used as the "standard" strain in all three designs, but other inbred (isogenic) mouse strains will be selected from those commercially available from Jackson Laboratories for studies in design 2. The BALB/C strain is also being used in the separate project being submitted to the Council by Dr. H. Herscowitz. Thus the results of his studies on the effects of cigarette smoke on alveolar macrophage function can be related to the results of the studies we propose.

2. Exposure to cigarette smoke.

Mice will be exposed to the smoke from University of Kentucky "reference" cigarette (1A1) in a prototype Walton Mark II horizontal smoke exposure machine. Cell and organ cultures will be exposed in the chamber recommended by Davies and Kistler (29) to allow adequte control of smoke mixing, temperature, humidity, etc. The dosage schedule will be determined in preliminary experiments after consultation with experts at the Council for Tobacco Research, U.S.A. Our aim is to duplicate the exposure of the "average" human smoker, as far as this may be possible.

3. Schematic presentation of the 3 major designs envisaged in this project are shown on the following 3 pages. In Design 1, pure populations of specific cell types of the alveolar wall from healthy mouse lung will be grown in culture and separately exposed in vitro to cigarette smoke. In Design 2, experimentally-induced and congenital variants in lung morphometry will be sought and the effects of such variants on lung metabolism and pathology studied. In Design 3, the effect of cigarette smoke on pre-and post-natal lung development will be studied by exposing pregnant females and neonates to smoke in vivo and by exposing organ cultures of developing lung to smoke in vitro.

It should be emphasized that the flow sheets outlining Designs I-III represent a concept of the experimental work we foresee. We do not propose that all of this work is to be accomplished within a five-year span. Rather, as certain

avenues of approach seem especially promising, they will be emphasized. Other less fruitful options may be dropped completely. We would envisage that the flow sheets will change each year as work progresses.

We should further emphasize that Project III is closely linked to Projects I and II in that the experimental material generated in III is utilized in both I and II. Similar cross utilization of material occurs within Project III itself, as indicated by the dashed arrows.

4. Narrative description of individual experimental design.
   a. Design I

Specific Background:

Investigation of the direct effects of cigarette smoke, or other environmental factors, on specific pulmonary cell types has been limited. However, a number of studies have been published on the direct effect of cigarette smoke, or its constituents, on the functions of the pulmonary macrophage (e.g. 30). A differential response of different lung cell types to cigarette smoke is implied by the pathological finding in different lung diseases that have been associated with cigarette smoking. For example, lung cancer is associated with stimulation of bronchial epithelial cells leading to hyperplasia and metaplasia. On the other hand, emphysema is characterized by degeneration of the aveolar walls. In vitro experiments using lung organ cultures (e.g. 32) have shown variations in cell response to cigarette smoke constituents, such as stimulation of epithelial proliferation and suppression of connective tissue cell type. In addition, the same cell type (ciliated bronchial epithelial cell) may exhibit altered susceptibility at different stages of development (32).

Previous in vitro studies have been directed towards detecting carcinogenic effects of components of cigarette smoke. Due to the high dosages used these studies do not provide satisfactory models for the chronic exposure of pulmonary cells to the levels of cigarette smoke, or air pollutants, found in the lungs of human smoker or non-smoker groups.

Procedures

a. Isolation of pulmonary cell types

Type II aveolar epithelial cells will be isolated by a modification of the procedure of Kikkawa and Yoneda (14) and used for direct biochemical analysis. The clonal isolation technique as used by Douglas and Kaighn (15) will be employed to derive cultures of functionally active type II cells. Cells from the peripheral tissue of healthy neonatal mouse lung will be disassociated by mild enzyme treatment and

cultured at very low density to favor the growth of single cell clones. Clones of epithelioid morphology which also exhibit large cytoplasmic granules when examined by phase contrast microscopy will be analyzed for ultrastructural evidence of lamellar bodies (2) and biochemical evidence of dipalmitoyl lecithin synthesis (see project 2). Clones exhibiting both these type II cell "markers" will be used for in vitro experiments.

Pulmonary endothelial cell cultures will be obtained by selecting clones with "endothelioid" morphology (18) and specifically identified by the detection of the following recognized "markers"; 1) Weibel-Palade bodies as demonstrable by EM (33) and 2) ability of the cells to convert Angiotensin I to Angiotensin II demonstrable by the release of tritiated His-Leu from ^{3}H-angiotensin I added to the culture (19,34).

Interstitial cell cultures will be derived by selecting "fibroblastic" clones and identified by demonstrating the presence of significant amounts of ^{3}H-hydroxyproline in protein secreted into the medium after exposure of the culture to ^{3}H-proline (20,35).

b. Exposure to cigarette smoke.

Replicate cultures of the individual cell types will be placed in the Davies and Kistler (29) chamber of the smoking machine and exposed to either 1) air, 2) whole cigarette smoke or 3) filtered cigarette smoke. Exposure will be arranged to be as closely analogous as possible to the exposure of cells in the distal airways of mice subjected to chronic smoking (see project I and Design II).

Dose response curves will be derived with respect to cell viability and plating (cloning) efficiency. Cultures exposed to non-toxic dosages will be examined for morphological and metabolic alterations (project 2) and, especially, any alterations in the expression of their "marker" functions.

Chronically exposed cultures will be analyzed periodically for possible progression of effects. Samples of such cultures will also be preserved in liquid nitrogen and thus will be available for further investigations.

DESIGN II

Specific Background

Breeding programs have resulted in the current availability of a large number of inbred (isogenic) mouse strains. Many anatomical variants in the various strains have been identified (36). No surveys for variants of lung morphometry have, so far, been undertaken.

With such excellent raw material and a practicable technique for morphometric screening, it is highly likely that strains showing congenital differences in the number of bronchial divisions can be identified for use in experimental study.

Chemicals have been used to modify the degree of bronchial branching in organ cultures of embryonic lungs (37,38). As there is extensive branching during postnatal lung maturation (27) the application of such chemicals to the lungs of neonatal mice may allow modification of lung maturation so as to provide morphometric variants with identical genetic

backgrounds.

Procedures

1. Mice of various inbred strains will be the subjects for lung morphometry described in the general methods section. Preliminary studies will establish the age (21 days; see ref. 27) at which all strains to be analyzed have completed post-natal lung maturation. Lungs of mice at this age (at least 3 litters, with at least 3 mice of each sex) will be analyzed to determine the average number of bronchial divisions and the variation a) between sexes, b) among mice of the same sex and c) between litters. Because of the isogenic nature of each strain, the latter two sources of variation should be minimal. The sample size may be altered depending upon the results of the preliminary statistical evaluation.

2. Newborn animals will be exposed to papverine (37), cytochalasin (see discussion by Wessells et al., 39) or proteolytic enzymes (40) which may be expected to impede or prevent subsequent bifurcation of developing lung epithelium. The method of exposure will be by allowing animals to inhale an aerosol containing the agent. The use of an inbred strain plus the prior establishment of intra-strain variance, makes use of litter mates of treated animals as controls quite valid.

3. From procedures 1) and/or 2) we expect to have groups of mice with lungs having low, intermediate and high numbers of bronchial divisions. Each group will be divided into: a) mice to be chronically exposed to cigarette smoke for a period of at least 6 months; and b) controls similarly treated but exposed to air only. At the end of this period analysis of lung function (project 1) and lung metablism (project 2) will be performed. The possible differential effects of cigarette smoke on mice with different lung morphometry will be ascertained.

DESIGN III

Specific Background

There is at present no integrated compilation of developmetnal data on the lung for any one animal model in terms of morphological, mophometric, physiological and biochemical parameters. The purpose of this part of Project 3 is to obtain such a profile of the properties of developing lung in BALBIC mice, and to determine whether exposure to cigarette smoke alters this development. As considerable and significant changes occur during the post-natal maturation of the lung (27), our study will encompass both pre- and postnatal development.

Further, fetal lungs from the three groups to be studied (see Procedures) will be explanted in organ culture at various developmental stages. Organ culture techniques are well established (42). Fetal lung has been grown in organ culture by Wolff[43], Taderera[44] and Chan et al[45] among others. The organ culture system provides for the use of conditions which are well defined by comparison to those extant in vivo. Thus, any influence of vitamins or hormones on response of lung tissue to smoke exposure could be

more directly measured. Further, in organ culture, three-dimensional relationships of tissues are maintained to a considerable degree. If bronchial branching is impeded or prevented, is there a modification of response to smoke exposure? Is the pattern of lung branching in vitro changed in lungs from fetuses whose mothers have been chronically or acutely exposed to smoke? Does smoke itself directly affect the branching activity of lung buds already in vitro? The in vitro system permits us to perform experimental manipulations which would be difficult indeed if attempted in vivo.

Another means of isolating fetal lung tissue from its normal environment to make it accessible to experimental manipulation is to graft it to the chick embryo choriallantoic membrane (CAM). Grafts of embryonic organs to the CAM of the chick embryo become vascularized and continue to develop with good maintanance of organ relationships. This technique has been used for years and is a simple one (reviewed in ref. 46). Mammalian organs are generally well maintained on the CAM. Larger rudiments can be grown than will survive in organ culture. Here then is a system in which the vascularized lung can be directly exposed to smoke. Morphometric analysis is quite feasible here. CAM grafts can also be used to monitor hormonal influences. Use of pituitaryless embryos (47) as hosts for the grafts provides a hormone deficient environment for the lung. Replacement therapy can be used to isolated individual endocrine effects.

Fetal lung grown either as a graft or in culture can be directly exposed to a branching inhibitor (in order to determine whether subsequent exposure to smoke results in an accentuated change in lung properties). However, the precise nature of the experments to be performed in organ culture or with CAM grafts will depend upon the in vivo findings in all three projects during the first 2 years of the program.

It should be reiterated here that Design III represents a large concept of experimental approach to the problem of analyzing lung development and some of the factors affecting this development. We do not imply that this entire Design will be accomplished within five years. Indeed certain lines of approach may well be found impractical and others not outlined here more meaningful. Thus, while the aims of Design III remain, the methods or lines of approach may vary.

Procedures

1. To study the possible effects of exposure to cigarette smoke on prenatal lung development, female BALB/C mice will be divided into three groups: chronic, acute and control. Chronic animals will be exposed to cigarette smoke for 21 days prior to mating. Exposure will continue throughout pregnancy. Acute animals will be exposed to smoke from day 1 of pregnancy and every day throughout pregnancy. Control animals will be handled in the same manner as experimentals but will be exposed only to air in the smoking apparatus.

It is recognized that every environmental parameter cannot be considered in every experimental design. However,

a few pilot studies will serve to decide whether total food intake should be monitored. Hence, one group of both control and acute animals will be given a diet corresponding to the amount consumed by the chronic group of animals. This dietary regime will commence in control and acute animals at the same time as the chronic group commences exposure to smoke. The results will help determine whether diet should be regulated in control and acute groups in later experments. This may be necessary if it is found that decreased food intake in the chronic group is responsible for any measured differences in the other groups.

Pregnancies will be terminated in three animals of each group on days 5, 12, and 17 (assuming a 19-day gestation). Data will be collected in the following format:

	Chronic			Acute			Control		
Day	5	12	17	5	12	17	5	12	17
# resoptions/horn									
# implants/horn									
position of implants in horn									
gross abnormalities									
wet wt. of each fetus*									
DNA/fetus*									

* These measurements will be recorded in terms of site of implantation in horn. DNA will be measured according to Schmidt (Colowick & Kaplan, Methods in Enzymology, Vol 3).

The data accumulated in this part of the design will establish a reliable baseline of fetal properties. This is important in understanding the variability in response to be expected from one experiment to the next. The following questions about fetal response to maternal smoke inhalation should be answered: a) do chronically exposed mothers produce litters with characteristics different from those of acutely exposed or control mothers (see methodology section)? b) Can severity of response be related to cumulative exposure time?

Lungs from fetuses of different and from newborn mice will be examined in terms of their morphological, morphometric (especially bronchial divisions) and metabolic (see Project 2) properties.

2. The effects of postnatal exposure to cigarette smoke on lung maturation will be studied by exposing litters of newborn mice to smoke daily during the period of maturation (23 days, ref. 27). At the end of this period the lungs of control and smoke-exposed mice will be analysed in terms of morphological, morphometric and metabolic properties.

3. To experimentally analyse factors involved in possible changes in fetal lung development associated with in vivo exposure to cigarette smoke, portions of fetal lung at various stages of development will be established in organ culture and/or as grafts to chick chorioallantoic membrane (CAM).

a) Organ culture techniques have been routinely used in our laboatory and technical details can be found in the references cited above. In brief, embryonic lung rudiments will be removed from the fetus under aseptic conditions, and placed in a culture dish, either on a semi-solid sub-

strate such as agar mixed with nutrient medium, or on a 'raft' floating on a liquid nutrient medium. The medium selected will be one that discourages outgrowth of cells from the organ explant and hence tends to maintain organ integrity. Development of the organ explants will be followed using the morphological and biochemical criteria described in Projects 1 and 2. Such cultures can be directly exposed to smoke and changes in lung properties attributable to smoke monitored.
b) Similarly, we have used the CAM grafting technique routinely. A small window will be cut through the shell and shell membrane of a 7-8 day old embryonated hen's egg. Lung buds removed from fetuses asceptically will be placed near a larger blood vessel on the CAM. The window will be sealed (scotch tape serves well) and the egg incubated until the graft has become well vascularized. For smoke exposure, or addition of chemicals, the window will be temporarily re-opened. If long term experiments are desired (longer than 10-11 days, at which time hatching of the chick would be imminent!), the graft will be removed at day 10-11 and re-passaged on a new 7-8 day old host.

1st Year: During the first year our major efforts will be devoted to 1) establishing cultures of functionally active alveolar cell types (Design I) and 2) establishing "baseline" data on the fetal developemnt of BALB/C mouse lungs and the influence of smoking on this development (Design III). We also plan to standardize the procedures for morphometric analysis of bronchial divisions on BALB/C mice (Design II).

2nd Year: We plan to undertake the experimental portion of Design I, analysing the effects of cigarette smoke on cultures of different cell types. When the analysis of fetal lung development is completed we will go on to study post-natal lung maturation and the effects of exposure to cigarette smoke upon this maturation (Design III, cont.). During the second year we also expect to be able to begin the morphometric survey of lungs in different inbred mouse strains (Design II).

3rd Year: We expect to concentrate on finishing the morphometric survey. If the first 4 inbred lines surveyed do not show any significant differnces in number of bronchial divisions we will turn to chemical modification of branching (Design II, part b) to obtain variants. The data on normal lung development that has been accumulated (from Projects 1,2 and 3) by this time should provide a good base for judging the effects of these chemicals on lung development.

4th Year: We hope to have groups of mice with lungs having respectively high, intermediate and low numbers of bronchial divisions. Chronic smoking of these mice will begin. During the fourth year we would also expect to use the cell cultures and/or organ cultures as models for experimentally testing the effects of other agents (e.g. vitamins, hormones, environmental pollutants) on lung cell function and/or lung developemnt in absence of, or presence of, cigarette smoke. The nature of these experiments will depend upon hypotheses generated from the data accumulated in all three projects of this program, as well as that reported by other investigators.

5th Year: Analysis of whether differences in the

number of bronchial divisions alters the susceptability of mice to chronic cigarette smoking should be possible. The results from Design II should provide more hypotheses to be tested in the "model" system that is most appropriate.

References

1. Weir, J.M. & M. Dunn. Cancer 25: 105 (1970).
2. Klaus et al. Science 137: 750 (1962).
3. Chevalier G. & A.J. Collett. Anat. Rec. 174: 289 (1972).
4. Page-Roberts, B.A. Biochim. Biophys. Acta 260: 334 (1972).
5. O'Hare, K.H. & P.L. Townes. J. Morhol. 132: 69 (1970).
6. Kaufman, S.L., P.H. Burri & E.R. Weibel. Anat. Rec. 180: 63 (1974).
7. Collet A.J.& G. DesBiens, Anat. Rec. 179: 343 (1974).
8. Emery, J.L. Resp. 27: (Suppl. p. 41) (1970).
9. Emery, J.L. & D.G. Fagan. Arch. Dis. Child. 45: 145 (1970).
10. Thurlbeck, W.M. Amer. Rev. Resp. Dis. 111: 803 (1975).
11. Vane, J.R. Brit. J. Pharmacol. 35: 209 (1969).
12. Scow et al. Lipids 7 : 497 (1972).
13. Hamosh, M. & P. Hamosh. Biochim. Biophys. Acta 380: 132 (1975).
14. Kikkawa, Y. & K. Yoneda. Lab. Invest. 30: 76 (1974).
15. Douglas, W.H. & M.E. Kaighn. In Vitro 10: 230 (1974).
16. Jaffe et al. J. Clin. Invest. 52: 2745 (1973).
17. Lewis et al. Science 181: 453 (1973).
18. Gimbrone, M.A. Jr., R.S. Cotran & J. Folkman. J.Cell Biol. 60: 673 (1974).
19. Ryan, J.W. & U. Smith. Prac. 20th Ann. Colloq. Protides of the Biol. Fluids p. 379 (1972).
20. Migra, T,. G. Martin & H. Eagle. Biochem. Biophys. Res. Comm. 53: 272 (1973).
21. Bradley, K., S. McConnell-Breul & R.G. Crystal Proc. Nat. Acad. Sci., USA 71: 2828 (1974).
22. Speizer, F.E. & B.G. Ferris. Arch. Environ. H th. 26: 313 (1973).
23. Hutchinson et al. Quart. J. Med. 41: 301 (1972).
24. Cederlof et al. Arch. Environ. Hlth. 18:934 (1969).
25. The Health Consequences of Smoking. A report of the Surgeon General. DHEW PUBLICATION No (HSM) 71-7513 p. 152 (1971).
26. Henderson, R., A. Hislop & L. Reid. Thorax 26: 195 (1971).
27. Thurlbeck, W.M. Amer. Rev. Resp. Dis. 111: 803 (1975).
28. Kullander, S. & B.A. Kallen. Acta. Obstr. Gynecol. Scand. 50: 247 (1971).
29. Davies, P. & G.S. Kistler. Experimentia 30: 436 (1974).
30. Green, G.M. Med. Clin. N. Amer. 57: 547 (1973).
31. Hamilton et al. Can. Med. Assoc. J. 77: 177(1957).
32. Lasnitzki, I. Cancer Res. 28: 510 (1968).
33. Weibel, E.R. & G.E. Palade. J. Cell Biol. 23: 101 (1964).
34. Soffer, R.L., R. Reza & P.R.B. Caldwell.
35. Peterkofsky, B. & D. J. Prockop. Anal. Biochem. 4: 400 (1962).
36. "Biology of the Laboratory Mouse" The Jackson Laboratory. Me (1941).
37. Ash et al. Develop. Biol. 33: 463 (1973)
38. Spooner B.S. International Conference on Lung Diseases, Montreal, Canada. May, 1975.

39. Wessells et al: Science 171:135 (1971).
40. Wessells, M. & R. Cohen. Develop. Biol. 18: 294 (1968).
41. Tjolue et al.Pharmacol. Taxicol. 26: 539 (1968).
42. Wessells, M. In Methods in Developmental Biology.(ed) Wilt, F.& N. Wessells. Crowell, N.Y. (1967). P. 445.
43. Wolffe, E. Develop. Biol. 3: 767 (1961).
44. Taderera, S. Develop. Biol. 16: 489 (1967).
45. Chan et al. Nature 228: 847 (1969).
46. Coulombre, A. In Metho ds in Developmental Biology. (ed) Wilt, F. & N. Wessells. Crowell, N.Y. (1967) P. 457.
47. Fugo, N. J. Exp. Zool. 85: 271 (1940).

ELECTRON MICROSCOPIC PROCEDURES

The methodology described below will be used in all three projects:

A. Ultrastructural Methods. Tissue samples from lungs in vivo, perfused lungs and in vitro cultured cells will be fixed in an aldehyde mixture (1) for two hours at 4°C and washed overnight in an appropriate buffer. Following the post-fixation in one percent osmium tetroxide, the samples will be dehydrated, embedded in Epon and sectioned with a diamond knife in 300-600 A° thin sections. Some grids will be treated conventionally with heavy metals, whereas the other grids will be processed according to phosphotungstic acid (10% aqueous solution, pH 1.5) procedure of Kay (2) and silver tetraphenylpophinesolfonate technique of Albert and Fleisbher (3) for demonstration for collagen and elastin fibers respectively.

B. Autoradiographic Methods (4). Tissue samples from lungs in vivo, perfused lungs and in vitro cultured cells will be fixed in a 2% osmium tetroxide buffer with veronal actate to pH 7.2 for two hours at 4°C and washed overnight in an appropnate buffer. Following the block staining in 1% uranyl actate, dehydration and embedding in Epon, tissue samples will be sectioned with a diamond knife in 600-900 A°thin sections and mounted on grids previously coated with collodion film baked by a thin carbon layer. The grids will be exposed to the photosensitive emulsion (Ilford L-4) diluted one to four with distilled water for approximately six months, developed in Microdal X for 5 minutes, washed, fixed in Kodak rapid fixer for 5 minutes and washed again in running and distilled water for 5 and 2 minutes respectively.

Electron microscopy and photogrphy will be carried out with the aid of an AEI-EM 8 electron microscope.

C. Preparation of Tissue for Scaning Electron Microscopy: Fixation in formaldehyde-glutaraldehyde 3% buffered to pH 7.2 with sodium cacodylate 0.1 M for 2 hours at 4°C will be followed by washing in buffer for up to 24 hours. The tissue will be then fixed in aqueous solution of 2% OsO4 for 1-2 hours and dehydrated by the critical point dry technique. Coating with a layer of ca 200A° of gold-parllodium will be the final step before observation with Etec Autoscane-B Scaning Electron Microscope.

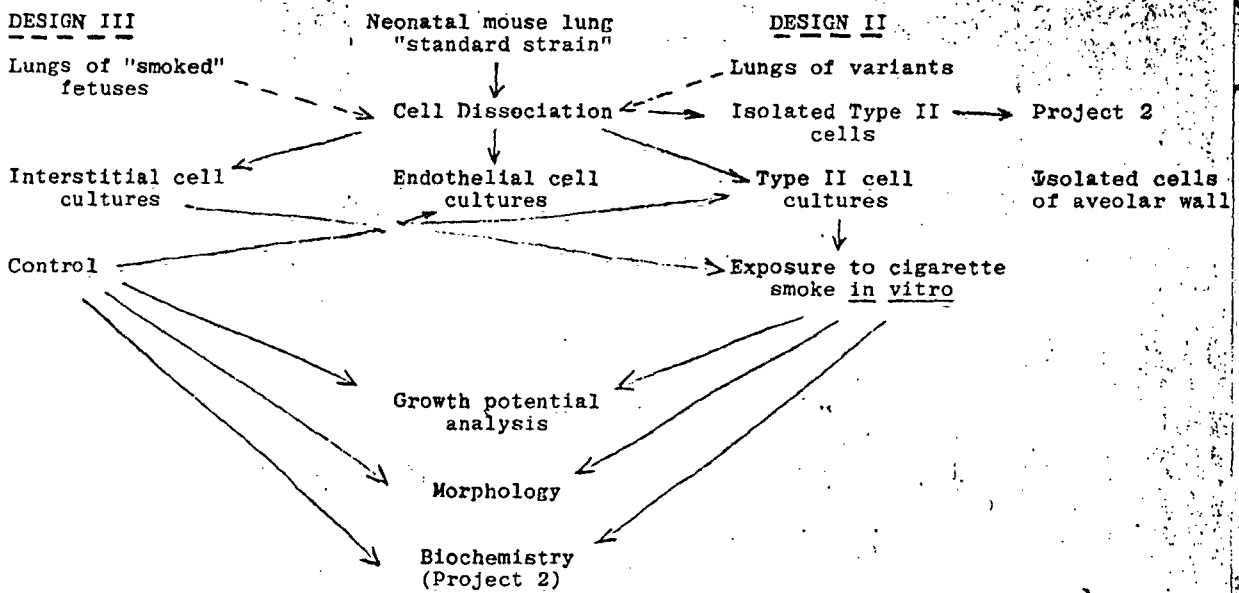
References

1. S. Ito and M.J. Karnovsky. J. Cell Biol. 39: 168A, 1968.
2. D.H. Kay. Tehniques for Electron Microscopy, II ed., F.A. Davis Co., Philadelphia, pp. 260-261, 1967.
3. E.N. Albert and E. Fleisbher. J. Histochem. Cytochem. 18: 697, 1970.
4. L.G. Caro, R.P.van Tubergen and J.A. Kolb. J. Cell Biol. 15: 173, 1962.

P. HAMOSH M.D.

PROJECT 3

DESIGN I



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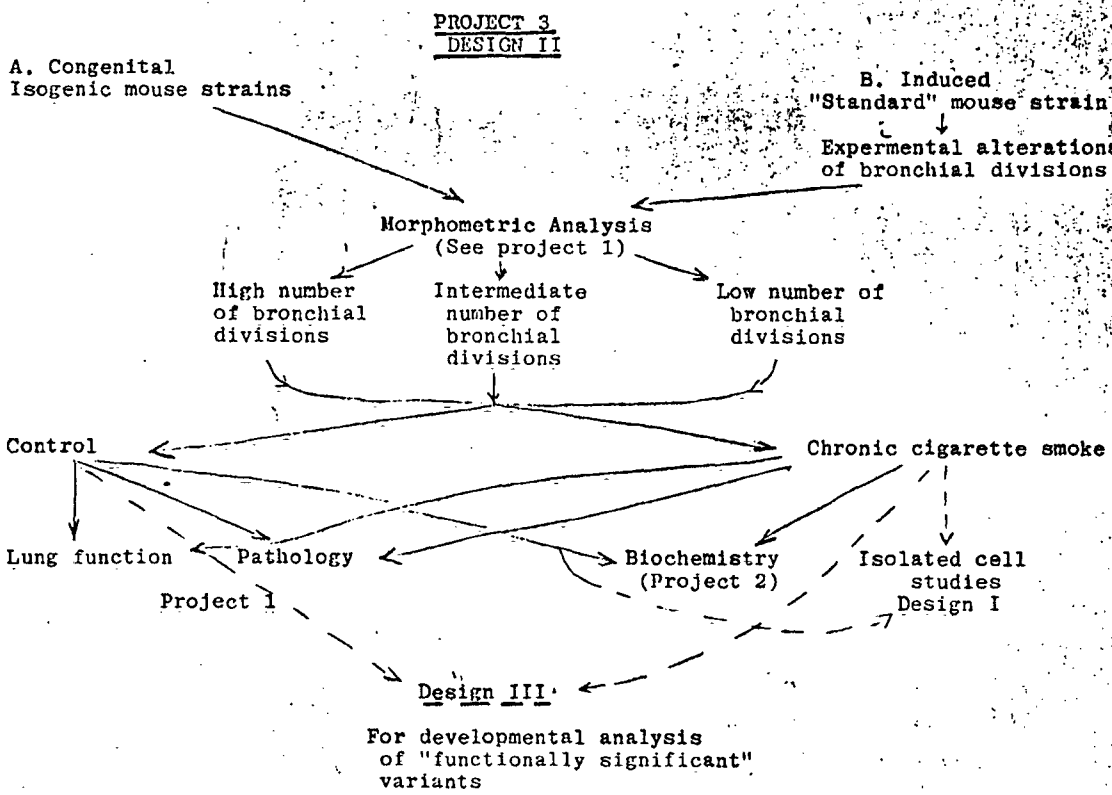
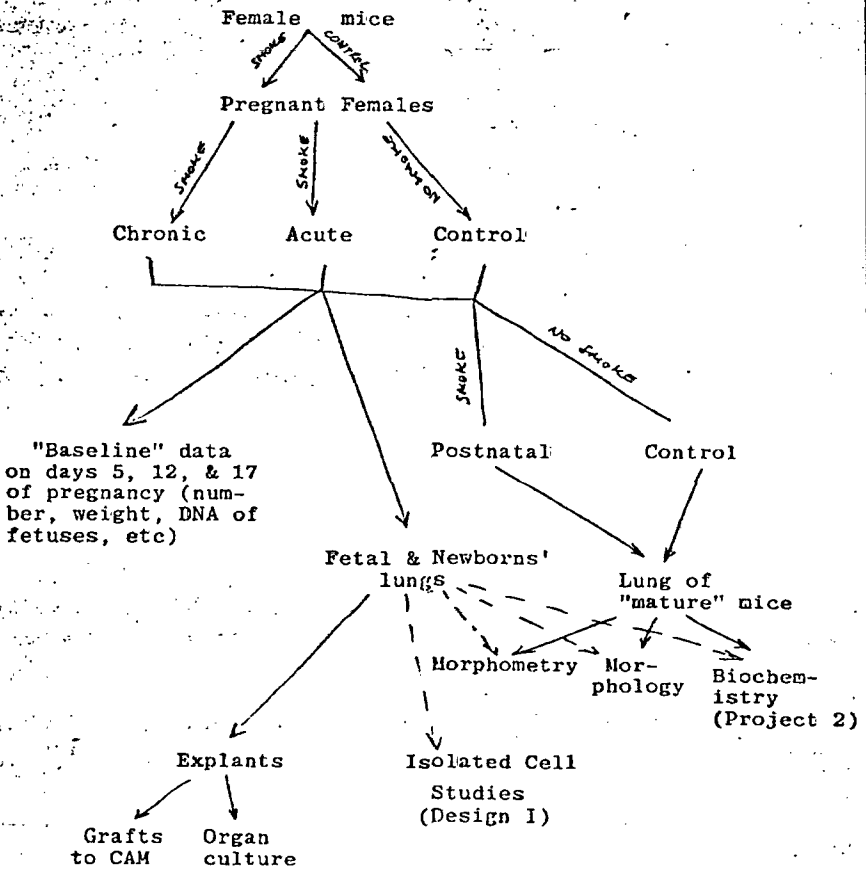


DESIGN III



First Year Budget
Project 3.

P. HAMOSH M.D.

3.

13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Jean Rew Wrathall Ph.D.	50%	REDACTED
Technical		
To Be Hired	100%	R
All Salaries Include 18.35% Fringe Benefits (16.7% 1/1/76-6/30/76 and 20% 7/1/76-12/31/76)	Sub-Total	REDACTED
B. Consumable Supplies (list by categories)		
Chemicals		2,500
Glassware and Disposables		2,500
Isotopes		2,500
Animals and Care		1,500
Surgical Instruments		1,000
	Sub-Total	10,000
C. Other Expenses (itemize)		
Computer Time		4,500
Travel		1,500
	Sub-Total	6,000
D. Permanent Equipment (itemize)		
Laminar Flow Hood		1,800
Co2 Incubator		1,300
Elutriation Rotor		6,500
Gradient Mixer and Pump		4,700
		14,300
E. Overhead (15% of A+B+C)	Total	52,722

Overhead to be negotiated at 15% or higher of A,B, & C

The distribution of effort by all investigators is presented in Figure 2

ok Hope
OK ARB

FACILITIES AVAILABLE: (Listed by Investigators)

Paul Hamosh, M. D. Department of Physiology and Biophysics
Space: Office, Secretary's office, laboratory for human studies, all purpose laboratory and laboratory for animal experiments (total area approximately 1000 square feet).

Space Shared: Departmental workshop, dark room, library and common office equipment room (copy machine, computer terminals and calculators).

Equipment:

Two 8 channel recorders - Hewlett-Packard (HP)
One x/y recorder HP
One 4 channel FM tape recorder HP
Four oscilloscopes, Tektronix
Twenty different amplifiers, HP, Validyne, Lexington, Tektronix
Two body plethysmographs with electronics
One total respiratory resistance unit, Lexington
One respiratory mechanics computer, Buxco
One blood gas analyzer, Corning
One bicycle ergometer, Quinton
Twenty transducers
Assorted animal respirators and plethysmographs complete electronic testing equipment, infusion and peristaltic pumps, etc.
Use of the facilities of the National Biomedical Research Foundation on a pay for services basis: an IBM 360/44 computer with all peripherals (see appended description).

Margit Hamosh, Ph.D.
Space: Office and Biochemistry Laboratory (approximately 250 square feet)
Space Shared: Additional laboratory space, cold room and conference room.
Equipment:

One spectrophotometer (Beckman 24)
One tissumizer (Tekmar)
One ultrasonic sonifier (Laboratory Supply Co.)
One driblock (Tecam)
IEC International centrifuge, Duenoff shaker, ovens, pumps, mixers, stirrers, chromatography equipment, etc.

Dr. Jean Wrathall, Ph.D.

Space: Tissue culture suite -approximately 800 square feet of space including 2 small "clean" rooms for aseptic transfers, a walk-in cold box, a small room with a chemical hood and 2 large rooms.

Equipment:

One dishwaser
One sterilizing oven
One Hotpak CO_2 forced-air incubator
One convection heated incubator
One table top clinicalcentrifuge
One Leitz microscope with objectives including oil immersion
One Olympus inverted microscope with conventional and phase contrast optics
One Cyrenco liquid nitrogen freezer
Two dissecting microscopes
One water bath
One magnetic stirrer
An autoclave is available in the Microbiology Department on the floor above.
Di-ionized water of satisfactory quality is available from the Microbiology Dept.

Dr. Gerald Goeringer, Ph.D.

Office Space:144 square feet

Laboratory space: 350 square feet

Equipment:

One Spinco Model L-4 ultracentrifuge with SW-65, SW-25 and 60 rotors
One International Model B-20 refrigerated centrifuge with 870 and swing-out rotors
One International Model H-12 table centrifuge with fixed and wing-out heads
One Zeiss model PMQII spectrophotometer
One ISCO UV Analyzer (in need of repair), Model UA-2
One EC 10 ml 20-tube counter-current train
One EC 20 ml 20-tube counter-current train
One EC Disc electrophoresis apparatus
One Buchler Model 3-1014A regulated voltage, current power supply
One Porter Blum Model MT-1 Ultramicrotome
One LKB Ultrorac Fraction Collector (in need of modification)
One Buchi Flash evaporator
One Precision Scientific Co. full view tissue culture incubator
One Labconco tissue culture hood

Goeringer continued

One New Brunswick controlled environment shaking incubator with 1 platform

One Zeiss photomicroscope II, Nomarski optics

One Zeiss Phase contrast microscope

One Heat systems Model W140 Ultrasonicator with 1 probe

One Sigmamotor peristaltic pump

One Photovolt Digicord pH meter

One Mettler H-16 Balance

One Mettler microbalance

One Mettler P-1000 toploading balance

One AO Stereomicroscope, Double head

One vacuum oven, vacuum pump, Virtis Lyophilizer (modified) (in need of repair)

One drying oven

Branislav Vidic, S.D.

Light Microscopy: Fully-equipped laboratory for processing of tissues (paraffin oven, hot plate), sectioning (microtome) and light microscopy (Zeiss binocular microscope with incorporated camera) are available in the Department of Anatomy.

Electron Microscopy: Fully-equipped laboratory for preparation of tissues, sectioning (diamond knives, LKB-ultramicrotome) and electron microscopy, transmission (AEI-EM 801) and scanning (ETEC AUTOSCAN B-1) are available on a part-time basis in the Department of Anatomy.

Major Facilities Required:

One Scintillation Counter

Two Smoking Machines

(The latter will be provided by the CTR on a lease basis).

Justification of Budgets

Project #1

1. Dr. Da Silva is joining on a fulltime basis the project in July 1976. Until then his support is assured from other sources. Projects A and D.
2. Ms. Linda Cooper has worked for the past year with project CTR #878. Project A and D.
3. A technician for histology to handle projects B and C, and projects 2 and 3 is included in full.
4. A second technician will be responsible to provide field support in project A and instrumentation support in all other projects.
5. Computer time is rated for 100 hours at $90. - (IBM 360/44).
6. Equipment: The system for the field station is an estimated price. We have several systems under investigation. A firm quote will be available later this year.
7. Other equipment is self explanatory and the estimates are based on previous work done for use at Johns Hopkins and Harvard Universities, whose craftsmen we frequently utilize.

Project #2

1. Dr. Margit Hamosh draws tentatively her entire support from this project. If additional sources now pending become available, this will be reduced.
2. Post doctoral fellows are being interviewed for this position, requiring some skill and experience.
3. A technician (probably Ms. Ruth Avigan) will provide technical support for this work.
4. Repair and Maintainance for service contracts on major equipment.
5. A new centrifuge is needed to replace an ailing international.

Project #3

1. Dr. Jean Wrathall will draw 50% of her support for the entire year.
2. The technician to be hired will assist in tissue culture work and in experiments performed by Drs. Wrathall and Goeringer.
3. Computer time is for morphometric work (50 hours).
4. Equipment. The hood and incubator are essential to upgrade the tissue culture suite. The rotor and mixer are used in cell separation.

Central Service and Facility

1. Part of Dr. Hamosh's salary is requested due to institutional guidelines.
2. The administrative assistant is co-ordinating all clerical work and bookkeeping and representing the program project vis à vis the administrative branches of the University (sponsored programs, purchasing, payroll, library, etc.).
3. The technician has dual function a) co-ordination of animal facilities, specimen flow and equipment maintainance and representation of the project vis à vis the technical branches of the University (housekeepping, engineering, supplies, etc.). b) provide "reserve" manpower, where most needed.

Equipment:

A scintillation counter is essential for the whole goup

(and Dr. Herscowitz). Presently the group is utilizing counter available by courtesy of their owners in Depts. of Physiology Pharmacology, Microbiology and Nuclear Medicine, with increasing volume of work, these arrangements will be insufficient.

Budgetary Considerations For Future Years

Whereas equipment expenditures are expected to decline in future years of support, expenses for salaries and supplies are expected to increase due to inflation and possible need of expansion. Budgets will be submitted for each fiscal year 6 months in advance. It is safe to assume that budget for the second year will remain about the same level as the first year budget and then increase at a rate of 5 to 10 percent.

SECTION II – PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 2, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Harosh, Paul, M.D.	Assoc. Professor of Physiology, Biophysics & Medicine	REDACTED
PLACE OF BIRTH (City, State, Country)	PRESENT NATIONALITY (If non-U.S. citizen, indicate kind of visa and expiration date) REDACTED	SEX ☒ Male ☐ Female

REDACTED EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
Hadassa, Hebrew University Medical School Jerusalem, Israel	M.D.	R	

HONORS

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Lung Mechanics and Metabolism	Responsible Investigator

RESEARCH SUPPORT (See instructions)

Principal Inv.: "The Effect of Smoking on the Small Airways". Grant #87 Council for Tobacco Research, Inc., U.S.A. $28,670 p/a (1/1/73 - 12/31/ effort-20%

Co-principal Inv. (with Dr. B. Vidic): "The Effect of Tobacco Smoke on Lung Metabolism." Grant # 936 Council for Tobacco Research, Inc., U.S.A. $40,000 p/a (1/1/74 - 12/31/76) effort-20%

Principal Inv.: "Chylomicrons and Atherosclerosis." Grant #802 Washingt Heart Ass. $11,615 (7/1/75 - 6/30/76) effort -10%

RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Starting with present position, list training and experience relevant to area of project. Li... or most representative publications. Do not exceed 3 pages for each individual.)

1975– Associate Professor of Physiology and Biophysics and Medicine Georgetown University Medical School, Washington, D.C.

1973-date Senior Cancer Research Internist, Veterans Administration Hospital/National Cancer Institute, Washington, D.C. (Part-ti

1973-date Special Lecturer in Child Health Care and Development and Be- havioral Sciences, George Washington University Medical Schoo Washington, D.C.

1973-1975 Assistant Professor of Medicine, Georgetown University Medica School, Washington, D.C.

1972-1975 Assistant Professor of Physiology and Biophysics, Georgetown University Medical School, Washington, D.C.

1970-1972 Director, Pulmonary Physiology Laboratory, Veterans Administration Hospital, Washington, D.C.

1970-1972 Assistant Professor of Medicine, George Washington University Medical School, Washington, D.C.

1968-1970 Research Associate, Veterans Administration Hospital, Washington, D.C.

Paul Hamosh Curriculum Vitae (Continued)

1968–1970 Lecturer in Physiology, Georgetown University Medical Schoo Washington, D.C

1966–1968 NIH Trainee in Cardio-Pulmonary Physiology, Department of Medicine, Georgetown University Medical School and Veterans Administration Hospital, Washington, D.C.

1963–1965 Department of Medicine B, "Ichilow" Municipal Hospital, Tel Aviv, Israel

1957–1963 Internship and Residency in Pathology, Medicine and Chest Diseases, Hadassa University Hospital, Jerusalem, Israel

PARTIAL LIST OF PUBLICATIONS

Dreyfuss, F., Hamosh, P. A coronary disease study among Cochin Jews in Israel. Am. J. Med. Sciences 240:769, 1960.

Dreyfuss, F., Hamosh, P., Adam, Y.G., Kallner, B. Coronary heart disease and hypertension among Jews immigrated to Israel from the Atla Mountain Region of North Africa. Am. Heart J. 62:470, 1961.

Hamosh, M., Hamosh, P., Bar-Maor, J.A., Cohen, M. Fatty-acid metaboli by human adipose tissues. J. Clin. Invest. 42:1648, 1963.

Zlotnich, A., Ramot, B., Hamosh, P. Hemoglobin-H disease with persistent hemoglobin "Bart's" in a Jewish family of Aleppo-Urfalian ancestry. Israel Med. J. 23:57, 1964.

Hamosh, P., Ramot, B., Molho, M. Primary hypoventilation syndrome of central origin. Israel J. Med. Science 2:448, 1966.

Ramot, B., Hamosh, P., Loeventhal, M.et al: Disseminated eosinophilic collagen disease. New Istanbul Contrib. Clin. Sc. 9:22, 1967.

Hamosh, P., Luchsinger, P.C. Maximum expiratory flow in isolated liquid-filled lungs. J. Appl. Physiol. 25:485, 1968.

Cohn, J.N., Khatri, I.M., Hamosh, P. Bedside catheterisation of the left ventricle in shock: a valuable diagnostic and therapeutic guide. Am. J. Cardiol. 25:66, 1970.

Hamosh, P., Luchsinger, P.C. Respiratory mechanics and gas exchange i the squatting position. Am. Rev. Resp. Dis. 102:112, 1970.

Hamosh, P., Cohn, J.N. Left ventricular function in acute myocardial infarction. J. Clin. Invest. 50:523, 1971.

Hamosh, P., Broder, M.I., Engelman, K., Cohn, J.N., Freis, E.D. Systo lic time intervals in acute myocardial infarction. Circulation 45:37 1972.

Gacad, G., Hamosh, P. The lung in ankylosing spondylitis. Am. Rev. Resp. Dis. 107:286, 1973.

Partial List of Publications, Continued

Da Silva, A.M.T., Hamosh, P. The effect of smoking a single cigarette on the small airways. J. Appl. Physiol. 34:361, 1973.

Hamosh, P., Da Silva, A.M.T. Supine hypoxemia and erythocytosis due to airway closure at low lung volumes. Am. J. Med. 55:80, 1973.

Hamosh, P., Da Silva, A.M.T. Air bolus method compared to single breath method for determination of closing volume. Am. Rev. Resp. Dis. 110: 518, 1974.

Hamosh, M., Hamosh, P. Lipoprotein lipase in rat lung, the effect of fasting. Biochim. Biophys. Acta. 380:132, 1975.

Hamosh, M., Hamosh, P. Effect of estrogen on lipoprotein lipase activity of rat adipose tissue. J. Clin. Invest. 55:1132, 1975.

Vidic, B., Hamosh, M., and Hamosh, P. Mucopolysacchrides in the pulmonary surfactant hypophase. An electron microscpical study of rat lungs treated with ruthenium red. J. Cell Biol. Submitted for publication.

Hamosh, P. The effect of shearing stress on the bronchial mucosa. Am. Rev. Resp. Dis. 109:694, 1974. (Abstract)

Vidic, B., Hamosh, M., Blunda, M., Hamosh, P. Effect of ventilation on uptake of ^{3}H-palmitate by isolated perfused rat lung. J. Cell Biol. 63:359a, 1974. (Abstract)

SECTION II – PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 3, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Hamosh, Margit, M. Sc., Ph.D.	Assistant Professor	REDACTED
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY** (If non-U.S. citizen, indicate kind of visa and expiration date)	**SEX**
REDACTED	REDACTED	☐ Male ☒ Female

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
Hadassa, Hebrew University Medical School Jerusalem, Israel	M. Sc. Ph. D.	R	Microbiology Biochemistry

HONORS

M. Sc. Summa Cum Laude

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Lipid Metabolism	Principal Investigator

RESEARCH SUPPORT (See instructions)

Co-Inv. : "The Effect of Tobacco Smoke on Lung Metabolism." Grant # 936 Council for Tobacco Research, Inc., U.S.A. $40,000 p/a (1/1/74 - 12/31/76)

Princ. Inv.: "The Effect of Estrogens on Human Adipose Tissue Lipoprotei Lipase Activity." Washington Heart Association # 690. $10,350 (1/1/75 - 12/31/75) effort - 10%

RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Starting with present position, list training and experience relevant to area of project. Li or most representative publications. Do not exceed 3 pages for each individual.)

1975-	Assistant Professor, Dept. of Anatomy, Georgetown University Medical School
1974-1975	Research Associate, Dept. of Anatomy, Georgetown University Medical School
1967-1974	Visiting Scientist, NIAMDD, Section on Endocrinology (R.C. Sc
1965-1967	Sabbatical leave, Visiting Scientist, NIMH, General & Comparative Biochem. (S. Kaufman)
1964-1965	Lecturer in Biochemistry, Hadassa, H.U. Medical School, Dept. of Biochemistry
1961-1964	Instructor in Biochemistry, Hadassa, H.U. Medical School
1959-1961	Post-Doctoral Fellow in Biochemistry, Hadassa, H.U. Medical School
1956-1959	Graduate Fellow in Biochemistry, Hadassa, H.U. Medical School

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213-54-6163

PARTIAL LIST OF PUBLICATIONS

M. Hamosh: Recent aspects on the pathogenesis of fever. Harefuah 58:376, 1960.

M. Hamosh and B. Shapiro: The localization of bacterial endotoxin in the organism. Dapim Refuiim 19:3, 1960.

M. Hamosh and B. Shapiro: The mechanism of glycogenolytic action of endotoxin. Brit. J. Exp. Path. 41:372, 1960.

E. Wertheimer, M. Hamosh and E. Shafrir: Factors affecting fat mobilization from adipose tissue. Am. J. Clin. Nutr. 8:705, 1960.

M. Hamosh and B. Shapiro: Lipid release by liver slices. Am. J. Physiol. 201:1030, 1961.

M. Hamosh, P. Hamosh, A. Bar-Maor and H. Cohen: Fatty acid metabolism by human adipose tissue. J. Clin. Invest. 42:1648, 1963.

M. Hamosh, R. Atia and B. Shapiro: Fatty acid uptake and esterification by fish muscle. J. Food Sci. 31:146-150, 1966.

M. Hamosh, M. Lesch, J. Baron and S. Kaufman: Enhanced protein synthesis in a cell-free system from hypertrophied skeletal muscle. Science 157:935, 1967.

M. Lesch, W. W. Parmely, M. Hamosh, S. Kaufman, E. H. Sonnenblick: The effects of acute hypertrophy on the contractile properties of skeletal muscle. Am. J. Physiol. 214:685, 1968.

Margit Hamosh and Robert O. Scow: Plasma triglyceride and mammary and adipose tissue lipoprotein lipase in pregnant and lactating rats. Yugoslav. Physiol. Pharmacol. Acta 6:169, 1970.

M. Hamosh, T. R. Clary, S. S. Chernick and R. O. Scow: Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim. Biophys. Acta 210:473, 1970

M. Hamosh and R. O. Scow: Lipoprotein lipase activity in guinea pig and rat milk. Biochim. Biophys. Acta 231:283, 1971.

R. O. Scow, M. Hamosh, E. J. Blanchette-Mackie and A. J. Evans: Uptak of blood triglyceride by various tissues. Lipids 7:497, 1972.

M. Hamosh and A. J. Evans: Lipoprotein lipase activity and triglyceride uptake in perfused adipose tissue. Fed. Proc. 31:297, 1972.

M. Hamosh and R. O. Scow: Lingual lipase and its role in the digestio of dietary lipid. J. Clin. Invest. 52:88, 1973.

O. Zinder, M. Hamosh, T. R. Clary-Fleck and R. O. Scow: Effect of

[redacted]

prolactin on lipoprotein lipase in mammary gland and adipose tissue of rats. Am. J. Physiol. 226:744, 1974.

M. Hamosh and P. Hamosh: Lipoprotein lipase in rat lung. Fed. Proc. 33:346, 1974.

W. A. Burns, M. J. Mathews, M. Hamosh, G. Vender Weide, and F. B. Johnson: Lipase secreting acinar cell carcinoma of the pancreas with polyarthropathy: A light and electron microscopic histochemical and biochemical study. Cancer. 33:1002, 1974.

B. Vidic, M. Hamosh, M. Blunda and P. Hamosh: Effect of ventilation on uptake of ^{3}H-palmitate by isolated, perfused rat lung. J. Cell Biol. 63:359A, 1974.

M. Hamosh, R. O. Scow, H. L. Klaeveman and R. O. Wolf: Role of pharyngeal lipase in lipid digestion in man. Clin. Res. 22:360A, 1974.

M. Hamosh and P. Hamosh: Lipoprotein lipase in rat lung, the effect of fasting. Biochim. Biophys. Acta 380:132, 1975.

M. Hamosh, H. L. Klaeveman, R. O. Wolf, and R. O. Scow: Pharyngeal lipase and digestion of dietary triglyceride in man. J. Clin. Invest. 55:908, 1975.

M. Hamosh and P. Hamosh: Effect of estrogen on lipoprotein lipase activity of rat adipose tissue. J. Clin. Invest. 55:1132, 1975.

M. Hamosh and A. R. Hand: Development of secretory activity in rat lingual serous glands. J. Dent. Res. 54:168, 1975.

CHAPTERS IN BOOKS

Margit Hamosh and Robert O. Scow: Plasma triglyceride and lipoprotein lipase activity in pregnant and lactating rats. Nutrition Proc. VIIIth International Congress of Nutrition, Prague 1969, Ed. J. Masek et al., Excerpta Medica, ICS No. 213, 207-209, 1970.

R. O. Scow, E. J. Blanchette-Mackie, M. Hamosh and A. J. Evans: Catabolism of plasma lipoproteins, in "Structure, metabolism, and clinical aspects of lipoproteins of blood." Wissenschaftliche Veroffentlichung der Deutschen Gesellschaft fur Ernahrung 23:100-114, 1973.

R. O. Scow, C. L. Mendelson, O. Zinder, M. Hamosh and E. J. Blanchette-Mackie: Role of lipoprotein lipase in the delivery of dietary fatty acids to lactating mammary tissue, in Dietary Lipids and Post Natal Development, Galli, C., Jacini, G. and Pecile, A. (Eds.) Raven Press, 91-114, 1973.

M. Hamosh and R. O. Scow: Lingual lipase, in Fourth Symposium on Oral Sensation and Perception, Ed. J. F. Bosma, 311-322, Fogarty International Center Proceedings, No. 21, 1974.

SECTION II – PRIVILEGED COMMUNICATION

BIOGRAPHICAL SKETCH

(Give the following information for all professional personnel listed on page 3, beginning with the Principal Investigator. Use continuation pages and follow the same general format for each person.)

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Vidic, Branislav, D. S.	Professor of Anatomy	REDACTED
PLACE OF BIRTH (City, State, Country)	**PRESENT NATIONALITY** (If non-U.S. citizen, indicate kind of visa and expiration date)	**SEX**
REDACTED	REDACTED	☐ Male ☐ Female

EDUCATION *(Begin with baccalaureate training and include postdoctoral)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	SCIENTIFIC FIELD
University of Belgrade, Yugoslavia	D.S.	R	Stomatology

HONORS

MAJOR RESEARCH INTEREST	ROLE IN PROPOSED PROJECT
Ultrastructure, glandular and pulmonary	Co-Investigator

RESEARCH SUPPORT *(See instructions)*

Princ. Inv.: "The Effect of Tobacco Smoke on Lung Metabolism." Council for Tobacco Research Inc., U.S.A. $936,340,000 (7/1/74-12/31/76) Effort - 25%.

Co-Inv.: Program Project, "Cellular and Immunological Bases of Pulmonary Disease." HL AI 16748-01 (5/1/74-4/30/79). Effort - 30%.

Princ. Inv.: "The Effect of Pulmonary Blood Flow on Surfactant Synthesis Washington Heart Association #752. $10,150 (1/1/75-12/31/75)

RESEARCH AND/OR PROFESSIONAL EXPERIENCE *(Starting with present position, list training and experience relevant to area of project. List all most representative publications. Do not exceed 3 pages for each individual.)*

Professor of Anatomy, School of Medicine and Dentistry, Georgetown University, Washington, D. C. 1974-present.

Associate Professor Anatomy, School of Medicine and Dentistry, Georgetown University, Washington, D. C. 1971-1974.

Associate Professor of Anatomy, School of Medicine and Dentistry, St. Louis University, St. Louis, Missouri. 1968-1971.

Assistant Professor of Anatomy, School of Medicine and Dentistry, St. Louis University, St. Louis, Missouri. 1965-1968.

First Assistant in Anatomy, School of Medicine, University of Lausanne, Switzerland. 1962-1965.

Visiting Assistant in Anatomy, School of Medicine, University of Basel, Switzerland. Summer, 1961.

Assistant in Anatomy, School of Medicine, University of Novi Sad, Yugoslavia. 1960-1962.

Demonstrator in Anatomy, School of Medicine and Stomatology, University of Belgrade, Yugoslavia. 1954-1958.

[redacted]

PARTIAL LIST OF PUBLICATIONS

The structure of the palatum osseum and its oral overgrowth. Acta Anatomica 71:94-99, 1968.

The sacral part of the sympathetic trunk in human fetuses and newborn. Archives d'Anatomie, d'Histologie et d'Embryologie 52:35-42, 1969 (W. Wozniak and B. Vidic).

The origin and the course of the communicating branch of the facial nerve to the lesser petrosal nerve in man. Anatomical Record 162:511-516, 1968.

Extreme development of the paranasal sinuses: Report of a case. Anna of Otology, Rhinology and Laryngology 78:1291-1298, 1969.

The communicating branch of the facial nerve to the lesser petrosal nerve in human fetuses and newborns. Archives d'Anatomie, d'Histologi et d'Embryologie 52:371-378, 1969 (B. Vidic and W. Wozniak).

The connections of the intra-osseous segment of the facial nerve in baboon (Papio sp.). Anatomical Record 168:477-490, 1970.

The morphogenesis of the lateral nasal wall in the early prenatal life of man. American Journal of Anatomy 130:121-140, 1971.

The prenatal morphogenesis of the lateral nasal wall in the rat (Mus rattus). Journal of Morphology 133:303-317, 1971.

The variations in height of the palatum osseum as a function of the variations of some other vertical dimensions and angles of the skull. Journal of Dental Research 50:14-17, 1971.

The intermediate root of the trigeminal nerve in the dog (Canis familiaris). Anatomical Record 169:697-703, 1971 (J. Augustine, B. Vidic and P. Young).

Metoda disekcije zdrela. Acta Medica Yugoslavica 24:241-250, 1970.

The histochemical and microscopical differentiation of the respiratory glands around the maxillary sinus of the rat. American Journal of Anatomy 132:491-514, 1971 (B. Vidic and H. Greditzer).

The respiratory glandular system in the rat lateral nasal wall in nor and polluted environments. Verhandlungsbericht des Anatomischen Gesellschaft, Anatomische Anzeiger 130:83-85, 1972 (B. Vidic, J. Taylor, M. Rana and B. Bhagat).

Comparative morphology of the communicating branch of the facial nerve Arkhiv Anatomii Gistologii Embriologii (Moscow) 62:17-21, 1972.

The dimensions and shape of the human maxillary sinus in the perinatal period. Acta Anatomica 83:411-415, 1972 (R. Cullen and B. Vidic).

The structure and prenatal morphogenesis of the nasal septum in the ra (Mus rattus). Journal of Morphology 137:131-149, 1972 (B. Vidic, H. Greditzer and W. Litchy).

SOCIAL SECURITY NUMBER [redacted]

The structure of the acinar cell and its relationship to the nerve terminals in the lateral nasal gland of the rat. Archivum Histologicu Japonicum 34:449-461, 1972 (B. Vidic and J. Taylor).

Functional behavior and structural modification of pulpal fibroblasts cultured in vitro. Archives d'Anatomie, d'Histologie et d'Embryologie 55:51-73, 1972 (B. Vidic, R. Chen and J. Taylor).

A reversible damage of the rat upper respiratory tract caused by cigarette smoking. Archives of Otolaryngology (B. Vidic, M. Rana and B. Bhagat) 99:110-113, 1974.

Structure and cytochemistry of the acinar cell in the rat maxillary gland. American Journal of Anatomy 137:102-117, 1973.

Uptake of marker particles by in vitro ventilated and perfused rat lung. American Journal of Anatomy 138:521-527, 1973.

Modifications, some cytochemical properties and transport of intra-lysosomal membranes. American Journal of Anatomy 141:361-373, 1974.

Effect of ventilation on uptake of ^{3}H-palmitate by isolated, perfused rat lung. Journal of Cell Biology 63:359A, 1974.

BOOKS AND CHAPTERS IN BOOKS

Manual of Human Dissection, St. Louis University Publication, St. Loui Missouri, 1970.

An Atlas of the Anatomy of the Ear, W. B. Saunders Co., Philadelphia, 1971 (B. Vidic and R. O'Rahilly).

Radiological Anatomy of the Nasal and Paranasal Cavities, W. H. Green, St. Louis, under consideration (B. Vidic, J. Fries and J. Martin).

An Atlas of the Anatomy of the Larynx, accepted for publication by W. B. Saunders Co., Philadelphia. To be published in 1975 (R. O'Rahilly, B. Vidic and J. Tucker).

Maxillary Sinus, in: Orban's Oral Histology, accepted for publicatio by Mosby Co., St. Louis. To be published in 1975.

Curriculum Vitae

Jean Rew Wrathall, Ph.D. Born: REDACTED

REDACTED

Education and Professional Experience:

Drew University, Madison, N. J.
University of Utah, Salt Lake City, Utah REDACTED
B.S. cum laude, 1964
Ph.D., 1969. Major: Genetics; Minor: Molecular Biology
Assistant Professor, Biology Department, State University of New York at Geneseo 1969-1970
Instructor of Genetics, Department of Obstetrics & Gynecology, Cornell University Medical College 1970-1972
Postdoctoral Fellow, Damon Runyon Memorial Fund for Cancer Research. Sponsor: Dr. Selma Silagi 1971-1973
Assistant Professor of Genetics, Department of Obstetrics & Gynecology, Cornell University Medical College 1973-1974
Research Associate, Department of Anatomy, Georgetown University School of Medicine, Washington, D. C. 1975-

Publications:

1. Silagi, S. and J.R. Wrathall. 1972. Suppression of tumorigenicity and tyrosinase activity with 5-bromodeoxyuridine. Internat. Cancer Conf. and VIIIth Internat. Pigment Cell Conf. Sydney, Australia, p. 91. (Abstract)

2. Oliver, C., J. Wrathall, S. Silagi and E. Essner. 1972. Suppression of tyrosinase activity and melanosome formation by BrdU in cultured mouse melanoma cells. J. Cell Biol. 55, 194a. (Abstract)

3. Silagi, S., D. Beju, J. Wrathall and E. deHarven. 1972. Tumorigenicity, immunogenicity, and virus production in mouse melanoma cells treated with 5-bromodeoxyuridine. Proc. Nat. Acad. Sci., USA 69, 3443-3447.

4. Wrathall, J.R., C. Oliver, S. Silagi and E. Essner. 1973. Suppression of pigmentation in mouse melanoma cells by 5-bromodeoxyuridine. Effects on tyrosinase activity and melanosome formation. J. Cell Biol. 57, 406-423.

5. Wrathall, J.R. and S. Silagi. 1973. Suppression of pigmentation in mouse melanoma cells by 5-bromodeoxyuridine: Effects on tyrosinase activity. Yale J. Biol. Med. 46, 427. (Abstract)

Curriculum Vitae

Born: REDACTED

REDACTED REDACTED

6. Schulman, J.D., J.R. Wrathall, S. Silagi and L. Doores. 1974. Altered amino acid concentrations accompanying suppression of malignancy by 5-bromodeoxyuridine. J. Nat. Cancer Inst. 52, 275-277.

7. Silagi, S. and J.R. Wrathall. 1974. Reversible suppression of differentiation and malignancy in bromodeoxyuridine-treated melanoma cells. In: Makahara, W., T. Ono, T. Sugimura and H. Sugano (Eds.) "Differentiation and Control of Malignancy of Tumor Cells." Univ. of Tokyo Press, Tokyo, pp. 361-385. Review

8. Wrathall, J.R., S. Silagi, R. Balint and E. Newcomb. 1974. Kinetics of suppression of melanoma cell tyrosinase activity and tumorigenicity by 5-bromodeoxyuridine. J. Cell Biol. 63, 379a. (Abstract)

9. Oliver, C., J. Wrathall, S. Silagi and H. Haimes. 1974. Effects of dexamethasone on mouse melanoma cells after long term culture in BrdU. J. Cell Biol. 63, 251a. (Abstract)

10. Wrathall, J.R. and S. Silagi. Suppression of melanoma cell tyrosinase activity after incorporation of 5-bromodeoxyuridine during one round of DNA synthesis. Proceedings of the IX International Pigment Cell Conference, S. Karger, Basal. In press (Article)

11. Silagi, S., E. Newcomb, J.R. Wrathall and J.K. Christman. Biochemical, immunological and morphological changes correlated with loss of tumorigenicity in 5-bromodeoxyuridine-grown melanoma cells. Proceedings of the IX International Pigment Cell Conference. S. Karger, Basal. In press (article)

12. Wrathall, J.R., E.W. Newcomb, R. Balint, L. Zeitz and S. Silagi. Suppression of melanoma cell tyrosinase activity and tumorigenic after incorporation of bromouracil for one or two cell divisions J. Cell Physiol. In press (article)

CURRICULUM VITAE

GERALD C. GOERINGER

Born : REDACTED

REDACTED

Education and Professional Experience: University of Pennsylvania Philadelphia, Pa. A.B. R (Major: Zoology; Minor: Chemistry

- Hahnemann Medical College Graduate School of Medicine Certificate, R (Isotope Methodology)
- Johns Hopkins University, REDACTED
- Marine Biological Laboratory, Woods Hole, Mass. Certificate, 1957 (Embryology)
- Northwestern University, Evanston, Illinois. United States Public Health Service Predoctoral Fellowship. Ph. D., 1959, (Embryology) Thesis: Modified Development of the Integument of Hypophysectomized Chick Embryos. I. The Epidermis. II. The Feather Germs.
- The Life Insurance Meidcal Research Fund. Postdoctoral Research Fellow, 1959-1962
- Research Associate in Immunochemistry at the Werner-Gren Institute, Stockholm, with Dr. Peter Perlman. 12 months, 1962-1963
- Assistant Professor of Biology, San Diego State College, San Diego, California, 1963-1966
- Assistant Professor of Anatomy, Georgetown University Schools of Medicine and Dentistry, Washington, D.C., 1966-1970
- Associate Professor of Anatomy, Georgetown University, 1970-date

List of Publications:

Some Attempts to Culture Aggregates of Dissociated Tubularia Cells in Glucose Solution. 1957, Marine Biological Laboratory Embryology Abstracts. Supported by Research Facilities Award, M.B.L.

The Effects of Sodium Cyanide and Ethyl Alcohol on the Polarity of of Regenerating Tubularia Stems. 1957, Marine Biological Laboratory Embryology Abstracts.

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CURRICULUM VITAE

GERALD C. GOERINGER

Born : REDACTED

Education and Professional Experience: University of Pennsylvania Philadelphia, Pa. A.B. R (Major: Zoology; Minor: Chemistry

- Hahnemann Medical College Graduate School of Medicine Certificate, 1955 (Isotope Methodology)
- Johns Hopkins University, R
- Marine Biological Laboratory, Woods Hole, Mass. Certificate, 1957 (Embryology)
- Northwestern University, Evanston, Illinois. United States Public Health Service Predoctoral Fellowship. Ph. D., 1959, (Embryology) Thesis: Modified Development of the Integument of Hypophysectomized Chick Embryos. I. The Epidermis. II. The Feather Germs.
- The Life Insurance Meidcal Research Fund. Postdoctoral Research Fellow, 1959-1962
- Research Associate in Immunochemistry at the Werner-Gren Institute, Stockholm, with Dr. Peter Perlman. 12 months, 1962-1963
- Assistant Professor of Biology, San Diego State College, San Diego, California, 1963-1966
- Assistant Professor of Anatomy, Georgetown University Schools of Medicine and Dentistry, Washington, D.C., 1966-1970
- Associate Professor of Anatomy, Georgetown University, 1970-date

List of Publications:

Some Attempts to Culture Aggregates of Dissociated Tubularia Cells in Glucose Solution. 1957, Marine Biological Laboratory Embryology Abstracts. Supported by Research Facilities Award, M.B.L.

The Effects of Sodium Cyanide and Ethyl Alcohol on the Polarity of of Regenerating Tubularia Stems. 1957, Marine Biological Laboratory Embryology Abstracts.

Modified Development of the Integument of Hypophysectomized Chick Embryos. I. The Epidermis. II. The Feather Germs. 1960, Dissertation Abstracts.

Antigens in Microsomes and other Subcellular Fractions of Parenchymal and Reticuloendothelial Cells of Rat Liver. Agar Diffusion Studies. 1964, in H. Peeters (Ed.), Protides of the Biological Fluids, 12th Colloquium, pp. 232-235. Elsevier, Amsterdam. (With P. Perlman and U. Lundkvist)

Parenchymal and Reticuloendothelial Cells of Rat Liver. Fluorescent Antibody Staining. 1964, in H. Peeters (Ed.), Protides of the Biological Fluids, 12th Colloquium, pp. 236- 241. Elsevier, Amsterdam. (With U. Lundkvist and P. Perlman)

Immunochemical Characterization of Parenchymal and Reticuloendothelial Cells of Rat Liver. 1966, Exp. Molec. Pathol. 5: 427-442. (With U. Lundkvist and P. Perlman)

Development of the Epidermis in Hypophysioprivic Chick Embryos. 1968, Anat. Rec. 160: 354.

Some Changes in Chorionic Epithelium undergoing Keratogenous Metaplasia. (With P.L. Morwood and T.M. Walker), 1969, Anat Rec. 163: 232-233.

Appearance of Enzyme Activity During Metaplastic Changes of the Chick Embryo Chorioallantoic Membrane. (With P.L. Morwood and T.M. Walker) 1970, Proc. Soc. Exp. Biol. Med. 134: 539-541.

Disc Electrophoresis of the Rat Oviduct and its Epithelium. 1971, Anat. Rec. 169: 325. (With C.W. Lynn).

Development of Epidermal Enzymes in the Hypophysectomized Chick Embryo Anat. Rec. (Accepted for publication) With P.L. Morwood.

The Role of the Pituitary in Development of Hydrolytic Enzymes in Chick Embryo Epidermis Transplanted to Chorioallantoic Membrane (With P.L. Morwood). In preparation.

Curriculum Vitae

Max Robinowitz, M.D.

Date of Birth:	REDACTED
Education:	Georgetown University, Washington, D.C., B.S. Honors in Biology. R
	Georgetown University, Washington, D.C., M.D. Cum Laude R
Training:	Barnes Hospital, St. Louis, Missouri, Straight Medicine Internship 1961-62; Medical Resident 1962-63.
	Mount Sinai Hospital, New York, New York, Pathology Resident 1963-67; Chief Resident in Pathology, 1965-67.
Military Service:	U.S. Army, Major MC, USAR, 1967-69; Walter Reed General Hospital, Washington, D.C. Assistant to Chief of Anatomic Pathology, 1967-68; Assistant Chief of Clinical Pathology, 1968-69.
Board Certification:	Anatomic and Clinical Pathology, 1967.
Experience:	Armed Forces Institute of Pathology, Washington, D.C., Assistant Pathologist, Obstetric, Gynecologic, and Breast Branch, 1969-70.
	Mount Sinai Hospital, Miami Beach, Florida, Associate Pathologist, 1970-72.
	Director, Radioimmunoassay Section, Georgetown University Hospital, 1972-1975.
	Staff Pathologist, Georgetown University Hospital (Anatomic and Clinical Pathology) 1972-
University Appointments:	Washington University School of Medicine, St. Louis, Missouri, Assistant in Medicine, 1962-63.
	Mount Sinai School of Medicine, New York, New York, Instructor in Pathology, 1965-67.
	University of Miami School of Medicine, Miami, Fla. Assistant Professor of Pathology, 1970-72.
	Georgetown University School of Medicine, Assistant Professor of Pathology 1972-

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Committees:

Interviewer for Georgetown University Medical School Admissions Committee, 1972-

Member, Georgetown University Medical Library Committee, 1972-

Member, Georgetown University Medical Center Laboratory Committee, 1972-

Teaching Appointments:

Georgetown University School of Medicine:

Faculty member, Sophomore Pathology Course for Medical and Dental Students

Faculty member, Introduction to Medicine Elective for Freshman students (weekly seminar)

Residency training in Anatomic and Clinical Pathology

Interdepartmental conferences with Medicine, Surgery and Pediatrics and Radiology.

Preceptor, Freshman Student Elective

Licensure:

Florida, District of Columbia, Maryland
Certification, National Board of Medical Examiners

Honors:

Alpha Omega Alpha:
Gold Medal in Medicine, Georgetown University, 1961.

Professional Societies:

REDACTED

REDACTED

Publications:

Rivera, R.A., McAllister, H.A., and Robinowitz, M., Fulminant hepatic necrosis following halothane anethesia, Med. Ann. Dist. Columbia, 39: 371-373, 1970.

Norris, H.J., and Robinowitz, M., Ovarian adenocarcinoma of mesonephric type, Cancer 28: 1074-1079, 1971.

Brief Report:

Robinowitz, M., Robinson, D.M. and Oppleman, P., Very Low Magnification Microscopic Technic, ASCP Summary Report 9:11, 1972.

Abstract:

Robinowitz, M., Mathew, J., Eckelman, W., and Harbert, J.C., Fatal reactions following ^{99m}Tc- ferrous hydroxide lung scan J. Nucl. Med. 14:445-446, 1973

91

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CURRICULUM VITAE

Name: Angelo N. Taveira da Silva, M.D.
Born: REDACTED
Sex: Male
Degrees: M.D. - Oporto Medical School
Oporto, Portugal. R

Research and
Professional experience: Portuguese Army 1963 - 1965

Oporto Medical School
Oporto, Portugal: Intern, 1966-1967

Jewish Hospital, Cincinnati, Ohio
Intern, 1967-1968

V.A. Hospital, Brooklyn, N.Y.
Resident in Internal Medicine, 1968-1970

V.A. Hospital (George Washington University, Washington, D.C.
Fellow in Pulmonary Diseases, 1970-1972

Oporto Medical School Hospital
Teaching Appointment 1972-1974

Georgetown University, Washington, D.C.
Research Associate, Physiology & Biophysics 1974-present time

National Board Status: FLEX - December, 1974

Medical Specialty Board Status: Diplomate American Board of Internal Medicine, 1972

Organizations: REDACTED

Publications:

1 - Da Silva, A. M. T., and Hamosh, P.: Effect of smoking a single cigarette on the "Small Airways". J. Appl. Physiol. 34:361, 1973.

2- Hamosh, P., and Da Silva, A. M. T.: Supine hypoxemia and erythrocytosis due to airway closure at low lung volumes. Am. J. Med. 55:80, 1973.

3- Hamosh, P., and Da Silva, A. M. T. : Air bolus method compared to single breath method for determination of closing volume. Am. Rev. Resp. Dis. 110:518, 1974.

92

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Smoking and the "small airways" P.I. Paul Hamosh	Council for Tobacco Research # 878	81,000	1/1/73-12/31/75
Smoking and Lung metabolism P.I. B. Vidic	CTR #936	140,000	1/1/74-12/31/76
The Effect of Pulmonary Blood Flow on Surfactant Synthsis P.I. B. Vidic	Washington Heart Association	10,350	1/1/74-12/31/75
Estrogen in Human Adipose Tissue Lipoprotein Lipase P.I. M. Hamosh	Washington Heart Association	10,350	1/1/74-12/31/75
The Role of Chylomicrons in Atherosclerosis P.I. P. Hamosh	Washington Heart Association	11,500	7/1/75-6/30/76
Cellular and Immunological Bases of Pulmonary Disease P.I. Joseph Bellanti	NHLI (HL 16748)	1,250,000	5/1/74-4/30/79

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Blood Triglyceride and Lipase in the Lung P.I. M. Hamosh (submitted)	National Heart and Lung Institute	140,540	1/1/76-12/31/78

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator.

Typed Name Paul Hamosh M.D.

Signature [signature] Date 6/30

Telephone REDACTED
Area Code Number Extension

Checks payable to:

Georgetown University

Mailing address for checks Sam A. Kimble
Georgetown University, 37th &O)
Sts. N.W. Washington D.C. 20007

Responsible officer of institution

Typed Name Sam A. Kimble

Title Administrator of Sponsored Programs

Signature [signature] Date 7/7/75

Telephone REDACTED
Area Code Number Extension

93

10035724